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INACTIVATION AND REACTIVATION OF XANTHINE OXIDASE IN DAIRY PRODUCTS

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SUMMARY

The effect of pasteurization, condensation, homogenization, and drying on xanthine oxidase (XO) has been studied. It has been found that heating whole milk to 195 F for 15 sec inactivates the XO, but when the same milk is condensed to 50% total solids and homogenized at 4,500 psi a portion of the XO is reactivated. More than 94% of the XO is inactivated when milk is exposed to sonic vibrations of a frequency of 10,000 vibrations per second for 7½ hr. The effect of mechanical stress applied to milk during processing on XO activity in dairy products is discussed. Data are presented which indicate that XO activity may be used to estimate the heat treatment a milk powder has received in processing.

Because of the demonstrated low order of specificity of xanthine oxidase (XO) allowing it to oxidize aldehydes, purines, and pyrimidines (3), and because of its known high concentration in milk (120 mg/liter) (4), a few efforts have been made to associate its activity with the oxidative deterioration of dairy products (1, 8, 14).

Although none of the results presented to date could be considered conclusive, they were sufficiently impressive to lead the authors into a study of XO activity in whole milk powders subject to flavor deterioration during storage. Since XO activity was found in powders made from milk which had undergone heat treatment sufficient to deactivate XO, a search was made for the mechanism responsible for its reactivation.

MATERIALS AND METHODS

Milk used in this study, produced by the Agricultural Research Center herd, was bulked and cooled rapidly to 45 F, and each morning was transported with a minimum of agitation to our pilot plant.

Foam-dried whole milk powders were made, using a slight modification of the vacuum drying process described by Sinnamon and his associates (15). The milk was standardized to 3.1% fat before processing into powder.

Nonfat milk powders were made using a 9-ft Swensen spray dryer[1] and employing conventional techniques. Where indicated, samples of nonfat powder were obtained from commercial sources.

XO activity was measured by using a variation of the method of Corran et al. (4). The time determined was that required to reduce, in an inert atmosphere, the methylene blue (MB) in a sample containing 8.6 ml of dairy product, 0.4 ml .0013 molar MB solution, and 1.0 ml of 0.4% hypoxanthine (HX) solution. The reagents were added in the sequence indicated and the reciprocal of the time interval from addition of HX to complete decolorization of the sample was used to express units of activity. XO activity was determined at 98.6 F.

Undenatured whey protein in nonfat powders was determined using the technique developed by Harland and Ashworth (9).

Heat treatment of milk samples was carried out on a laboratory scale using stainless steel containers and steam heating. Total time elapsed during bringing samples up to temperature and cooling was approximately 1 min.

Pilot plant scale heating was done by using either a Cherry-Burrell Model CC35 spray pasteurizer[1] or a Mallory type heater.[1]

Where required, milk samples were concentrated using a Rogers single-effect pan[1] and homogenized using a Manton-Gaulin Model K3-75 homogenizer.[1] Milk samples were subjected to sonic vibration in a Raytheon[1] 10KC-250 watt sonic oscillator. Temperature was maintained at 34 to 36 F during the irradiation period.

[1] The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

RESULTS

On averaging results from ten samples of milk, the respective XO activities before and after pasteurization, and per cent inactivation by pasteurization, were: for whole milk (4.1% fat) 21.7, 16.6, and 23.6%; cream (40.3% fat) 257.8, 251.9, 2.3%; and skimmilk (0.12% fat) 7.2, 5.2, 27.9%.

Figure 1 graphically presents the effect of extended heat treatment at 170 F on the XO activity of whole and nonfat milk, and the cream obtained therefrom. The stabilizing effect of fat is again noted and XO activity could still be detected in whole milk after 15 min of heating at this temperature.

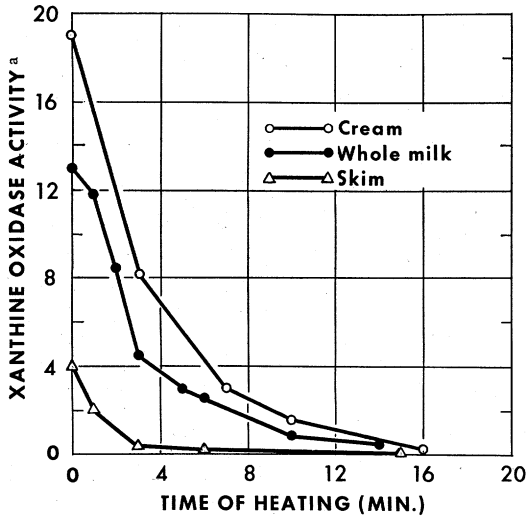

FIG. 1. Inactivation of xanthine oxidase at 170 F.

[a] Unit scale for skim and whole milk, × 10; cream, × 100.

To check reproducibility of these laboratory investigations when using pilot plant equipment, a study of the effect of heat on XO activity was made using holder and high temperature–short time (HTST) heating equipment.

Table 1 sums up the pertinent observations on XO inactivation during pilot plant heat treatments. The results of pasteurization using a spray pasteurizer were similar to those obtained using laboratory apparatus. However, far greater loss than anticipated was noted in the XO activity of the milk passed through the Mallory type heat exchanger. In fact, samples heated at 195 F for 15 sec exhibited no detectable XO activity.

Vacuum foam-dried whole milk powders were made from milk pasteurized by using both holder and HTST techniques. Table 2 summarizes the measurements of XO activity in the initial heat treated milk and in the reconstituted powder. The apparent partial reactivation of XO activity during the production of milk powder made from milk heated at 195 F for 15 sec was further investigated by following the XO activity change during each step of the production sequence.

TABLE 1

Inactivation of xanthine oxidase effected by various time-temperature combinations employed in pasteurization

Type of heats	Pasteurization temperature	Pasteurization time	XO activity	Pasteurization inactivation
	(F)	*(sec)*	*(units × 10)*	*(%)*
None			20.0	0.0
Spray pasteurizer	145	1,800	12.5	37.5
Mallory	165	15	8.3	58.4
Mallory	195	15	0.0	100.0

Concentrating heat-treated milk in a single effect pan to 50% solids content was found to have little or no effect on XO activity. However, homogenizing the 50% concentrate at 4,500 lb pressure was found to reactivate part of the XO. The results of a typical experiment are shown in Table 3. This reactivation was not observed in powders made from milks heated in excess of 200 F for 15 sec. Neither did increased homogenization pressure reactivate increased amounts of XO, as shown in Table 4.

The demonstrated sensitivity of XO to mechanical stress was further studied by subjecting samples of milk to sonic vibrations. Table 5 tabulates the loss in enzyme activity experienced during an extended irradiation period.

The residual XO activity in spray-dried nonfat powders was determined using pilot plant and commercially produced samples. From the

TABLE 2

Xanthine oxidase activity in heat-treated milk and the whole milk powders made therefrom

Milk heat treatment employed	XO activity		Change of XO activity during processing
	(units × 10)		*(units × 10)*
	Milk	Powder	
145 F—30 min	13.0	12.7	− .3
165 F—15 sec	12.2	11.6	− .6
195 F—15 sec	0.0	2.0	+2.0

TABLE 3

Effect of homogenization at 4,500 psi on xanthine oxidase activity in 50% solids of concentrates made from milk pasteurized at different temperatures

Pasteurization treatment	XO activity before homogenization	XO activity after homogenization	Change in XO activity during homogenization
	(units × 10)	*(units × 10)*	*(units × 10)*
145 F—30 min	13.3	12.8	− .5
165 F—15 sec	11.6	11.6	−0.0
195 F—15 sec	0.0	1.7	+1.7

results presented in Table 6, it can be seen that commercial high heat powders have zero XO activity. The decrease in undenatured whey protein roughly correlated with a decrease in XO activity. The XO activity may possibly be used as a simple rapid method of estimating the heat treatment a nonfat milk had received as a result of drying.

DISCUSSION

Morton (11) demonstrated that the enzyme XO is found in fresh milk in the form of lipid-protein complexes occluded onto the surface of the fat globules. These complexes, known as microsomes or liposomes are thought to arise from the microsomes released by the secretory cells of the mammary gland. On incubation in milk serum, during the periods between milkings, the cellular microsomes lose nucleic acid and lipids and gain in XO to acquire the characteristics of the microsomes found in milk (2).

A study of the effect of activation on rates of oxidation of aldehydes by XO gives further evidence of the complexity in depth of the fat-water interface (13). Activation of the enzyme to its less specific form is thought to be accomplished by disruption of the fat globule microsome bonds and eventual solution of the monodisperse enzyme in the aqueous phase of milk. This dispersion and subsequent activation of the enzyme can be effected by heat and mechanical stress. Polonovski and his associates (12) have carried out basic studies on the effect of heat, pressure, ultrasonic vibration, and surface active agents on the activity of XO in milk. In these studies it was clearly demonstrated that those physical factors capable of activating XO could also destroy its activity if applied at high levels over extended periods of time.

In view of the involved pattern of the physical forces milk encounters in its passage through equipment used in dairy product manufacture, the authors of this paper were particularly interested in the translation of laboratory results to pilot plant operations. The

TABLE 4

Effect of the pressure of homogenization on reactivation of XO in 50% solids concentrate made from milk pasteurized at 195 F for 15 sec

Homogenization pressure	XO activity	Change in XO activity
(psi)	*(units × 10)*	*(units × 10)*
Control	0.0	0.0
4,500	1.8	+1.8
6,000	0.1	+0.1
8,000	0.0	0.0

TABLE 5

Inactivation of XO activity in milk by sonic waves vibrating at 10,000 cycles per second

Sample no.	Time of exposure to vibration	XO activity	XO inactivation
	(hr)	*(units × 10)*	*(%)*
Control	0	22.2	0.0
1	2	7.0	63.8
2	4	2.7	88.0
3	6	1.3	93.9
4	7.5	1.0	95.4

TABLE 6

Xanthine oxidase activity in dried skimmilk samples

Milk preheating temperature	Milk preheating time	Undenatured whey protein nitrogen	Xanthine oxidase activity
(F)	*(min)*	*(mg/g)*	*(units × 10)*
Raw	....	7.8	5.5
150	30	7.0	3.9
160	30	5.6	2.2
170	30	3.2	0.0
180	30	1.6	0.0
185+	30	0.4	0.0
170+	0.33	5.7	4.0
165*	0.25	5.3	4.3
210*	10.00	0.4	0.0
215′	0.25	1.0	0.0
160′	0.50	6.8	4.5

+, *, ′ Samples with the same sign were made by the same manufacturer.

influence of processing on the residual XO content of dairy products was in some instances different from that anticipated from a simple extrapolation of a set of experimental data involving only single activation or deactivation factors.

On considering the data presented in this paper, and the papers referred to, it can be concluded that physical forces acting singly or in conjunction with one another radically alter the XO activity of milk.

While low levels of heat tend to activate XO by increasing the degree of dispersion of the enzyme, the free and active enzyme is apparently sensitive to heat deactivation. Since high pressures also tend to disrupt the stabilizing microsome-fat globule interaction, HTST heat exchangers produce more XO deactivation than would be anticipated from laboratory heating studies. Those exchangers subjecting the milk to the maximum pressure, turbulence, and vibration during the heating cycle could be expected to reduce XO activity to the greatest extent.

Even though many of the observed changes in XO activity in milk can be explained on the basis of increased dissociation of the enzyme-lipid complex, the observed partial reactivation of XO in HTST heat-treated milk concentrates lacks satisfactory explanation. Since increasing the homogenization pressure does not increase the amount of activation, it may be possible that two forms of xanthine oxidase are present in milk, one form of the enzyme being stabilized in an inactive form by a very close association with the fat globule. High-pressure homogenization of high solids concentrates promotes a casein-fat interaction (6) which may displace the enzyme from the fat surface, thereby activating it. Excessive homogenization pressures, therefore, not only would free the enzyme but rapidly denature its active and pressure sensitive form. This deactivation was observed at pressures in excess of 4,500 lb homogenization.

These observations tend to bear out the fact that the physical stress applied to milk in production equipment should be considered as a means of product quality control.

The exact results of the poorly recognized changes effected by simple physical forces on product quality are largely unknown. Therefore, until adequate research is carried out, some caution should be exercised in projecting laboratory findings directly to pilot plant or commercial operations. Expectations of reproducibility of the results of similar production sequences carried out using equipment of different design also seem unwarranted in light of the reported study.

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